

**W. P. Müller, Ch. H. Thiébaud, L. Ricard and M. Fischberg. —  
The induction of triploidy by pressure in *Xenopus laevis*.****ABSTRACT**

Hydrostatic pressure has been used to induce triploidy in *Xenopus laevis*. Five minutes after artificial insemination the eggs were pressurized for 5 min. The best results were obtained at pressures of 400 and 450 at when up to 97% triploids were found amongst the normal embryos and tadpoles. This method is compared with temperature shock experiments of *X. laevis* eggs and to results obtained by subjecting *Rana pipiens* eggs to pressure.

**INTRODUCTION**

Newly spawned amphibian eggs are generally at metaphase of the second meiotic division. The entry of the sperm induces the completion of the second meiotic division which is followed by extrusion of the second polar body. Suppression of the formation of the second polar body leads to a triploid embryo possessing two maternal and one paternal set of chromosomes.

The formation of the second polar body can be suppressed by treatment of the eggs with cold or hot temperatures or by hydrostatic pressure. Treatment with low temperatures (0-4° C) results in a rather low incidence of triploidy while treatment with 35.5-36.5° C, yields a higher incidence of triploidy but is accompanied by a high lethality.

The eggs of the tropical anuran *Xenopus laevis* do not survive treatment with 0°-4° C, however, 54% of the surviving tadpoles are triploid after treatment with 36.1-36.5° C. The remaining 46% possessing various chromosome numbers ranging from haploids and diploids to tetraploids, aneuploids and various chromosome number mosaics (SMITH, 1958). This fact renders the selection of triploids very uncertain and time consuming. In order to improve the yield of triploids amongst the surviving tadpoles we applied hydrostatic pressure to the eggs of *Xenopus laevis*, following more or less the method used by DASGUPTA (1962) for *Rana pipiens*.

**MATERIALS AND METHODS**

*Artificial insemination* of the eggs is advantageous in order to allow precise timing and the simultaneous treatment of many eggs. The insemination was carried out according to WOLF and HEDRICK (1971). Three females and two males were used. Eggs from ♀ 1 and ♀ 2 were inseminated with sperm from ♂ 1, eggs from ♀ 3 with sperm from ♂ 2. Some 30 to 100 eggs at a time were placed in a plastic petri dish of 2.5 cm diameter which was then introduced into the pressure chamber.

*Hydrostatic pressure* is provided by a "HEIN WERNER" hydraulic press which acts on a pressure chamber formed by a hollow stainless steel cylinder (12 cm long with an

outer diameter of 7 cm, a bore of 9.5 cm in length and a diameter of 3 cm). A roof shaped joint (Merkel automatic No. 1160-014 35 mm Type E 235 45 × 30 mm) fitted at 45 mm from the bottom of the bore provides good tightness. A 12 cm long stainless steel piston with a copper mantle piece and a concave end is introduced into the pressure chamber

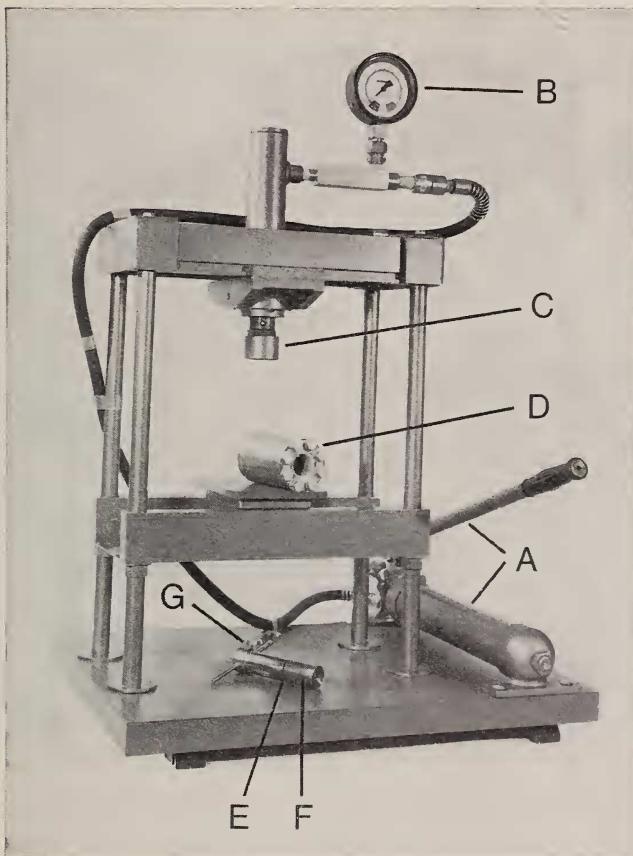


FIG. 1.

The pressure apparatus composed of a "HEIN WERNER" hydraulic press  
(A = pump; B = manometer; C = piston)  
and the pressure chamber = D with its piston = E  
its copper mantle = F and the screw valve = G.

(fig. 1). A screw valve, open when introducing the piston, permits the evacuation of the air and the supplementary water through a longitudinal canal. After closing the screw valve pressure was applied 5 minutes after insemination for a period of 5 minutes. Pressures of 280 at, 340 at, 400 at and 450 at were used.

*Determination of ploidy:* In *Xenopus laevis* each haploid chromosome set possesses one recognizable chromosome with a nucleolar organizer. The maximal number of

nucleoli found in a cell population thus corresponds in principle with the degree of ploidy (fig. 2). At the stages used (embryos and tadpoles) a diploid individual possesses 2 nucleoli in about 65% of its nuclei and 1 (fused) nucleolus in about 35% of its nuclei. The figures for triploid embryos and tadpole tails are about 30% of cells with 3 nucleoli, 50% with 2 and 20% with 1 nucleolus. A strong deviation from these proportions

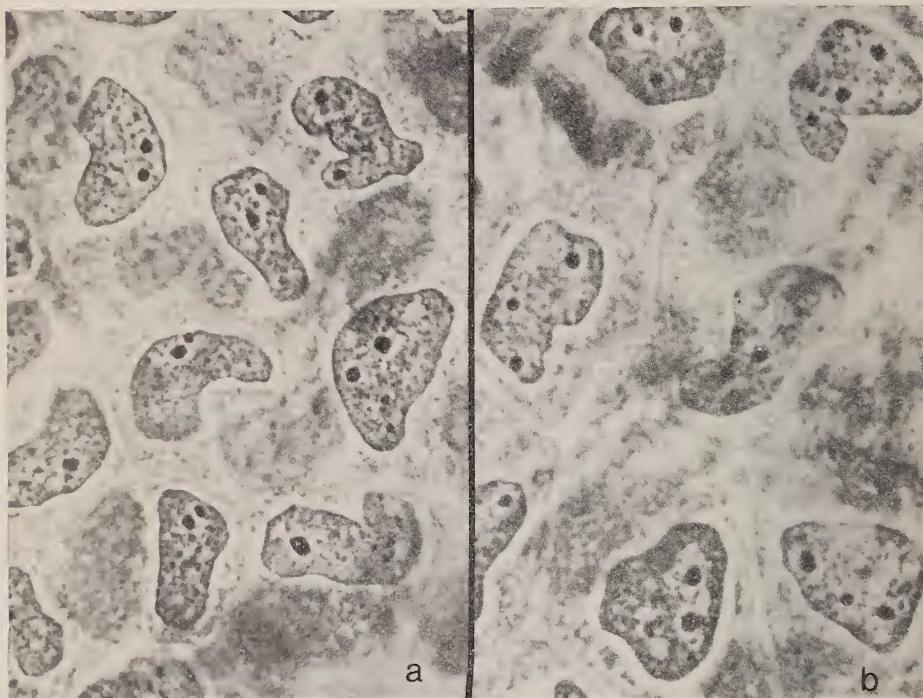


FIG. 2.

The number of nucleoli in epidermal cells of the tail (total preparation) of *Xenopus laevis*.

a) of a diploid tadpole and b) of a triploid tadpole.  
Phase contrast  $\times 250$  original.

indicates chromosome number mosaics. We used this rapid and well-known method (see FANKHAUSER 1955 and SMITH 1958) in order to determine the ploidy of larger samples for each pressure and the progeny of each female used. Whole embryos or tails of tadpoles were slightly squashed and the nucleoli of 100 cells per individual counted. Moreover, for eight of them the DNA content of 100 erythrocyte nuclei per tadpole was measured according to THIÉBAUD and FISCHBERG (1977) and for three of these the triploidy was confirmed by chromosome counts (fig. 3), using the technique of TYMOWSKA and KOBEL (1972). The degree of ploidy of 25 control embryos or tadpoles per female used was determined in order to exclude the possibility of a massiv production of spontaneous triploidy as is sometimes the case for a particular female.

## RESULTS

Table 1 summarizes the results obtained after treatment with different pressures. It also shows that all the control eggs of the three females developed into diploid embryos or tadpoles. There was 5.3% abnormal development amongst the control eggs whilst in the experimental series this value varied between 0-38% with an overall average of 23.8%. Within the limits of the experiment the percentage of abnormals was independent of the pressure used, but varied from female to female and even for the same female from one batch of eggs to another. Diploid and triploid embryos and larvae were about equally numerous amongst the abnormals. As expected most haploids, higher polyploids (4-6 N) and chromosome number mosaics were found amongst the abnormals. At all the pressures used a few haploid, a few tetraploid, pentaploid and hexaploid embryos and larvae were induced. The higher polyploid individuals seemed to become more numerous as the pressure increased. A few chromosome number mosaics were also present. The proportion of triploids increased clearly with the pressure used to reach about 90% of all the eggs treated at 400 and 450 at. The diploid embryos and larvae still quite numerous at 290 at were almost entirely absent at 400 and 450 at.

The value of our ploidy determination by nucleolar counts was tested, *a*) by chromosome counts of 3 tadpoles possessing 3 nucleoli, 54 chromosomes were found instead of the diploid number of 36 (fig. 3*a*, *b*). The number of evaluated metaphases for the 3 tadpoles was 26, 11 and 5 respectively. *b*) by comparing the DNA-content of 100 erythrocyte nuclei for each of 8 three-nucleolated tadpoles with that of 100 erythrocyte nuclei of each of 3 two-nucleolated individuals (method of THIEBAUD and FISCHBERG 1977). The mean DNA content in % of the diploid value was  $150.1 \pm 0.6$ , thus confirming the presence of an additional haploid set of chromosomes. The nucleolar counts, as performed by us (maximal number of nucleoli and their proportion in % of the cell population checked) result, therefore, in a sufficiently accurate determination of the ploidy.

## DISCUSSION

Hydrostatic pressure is an excellent tool to induce triploidy in Amphibia. Amongst normally developing embryos, DASGUPTA (1962) obtained for *Rana pipiens* 85% triploids under optimal conditions. We obtained for *X. laevis* a yield of 97% triploids amongst the normal embryos and tadpoles. The optimal pressure for the production of triploids in *Rana pipiens* is 350 at, a higher pressure of 420 at being lethal. In *Xenopus laevis* the best results were obtained at pressures of 400 and 450 at. A higher pressure has not been used to avoid possible damage to the apparatus.

The use of hydrostatic pressure has certain advantages as compared to temperature shock. The lethality of the eggs and the number of abnormal embryos is lower and the percentage of triploids (97%) is considerably higher.

No delay of development was observed after exposing *X. laevis* eggs to pressure at 20.5° C, while DASGUPTA (1962) noted a delay of 10-15 min. between insemination and first cleavage in pressurized *Rana pipiens* eggs as compared to their controls.

As in *Rana pipiens*, in *Xenopus laevis* a low percentage of other heteroploid embryos and tadpoles was found in addition to the triploids after exposing the eggs to pressure. Amongst these haploids (probably after loss of all egg chromosomes) were the most frequent. The possible origin of the few tetraploids and hexaploids and the more frequent pentaploid, aneuploid and chromosome number mosaic embryos shall not be explained

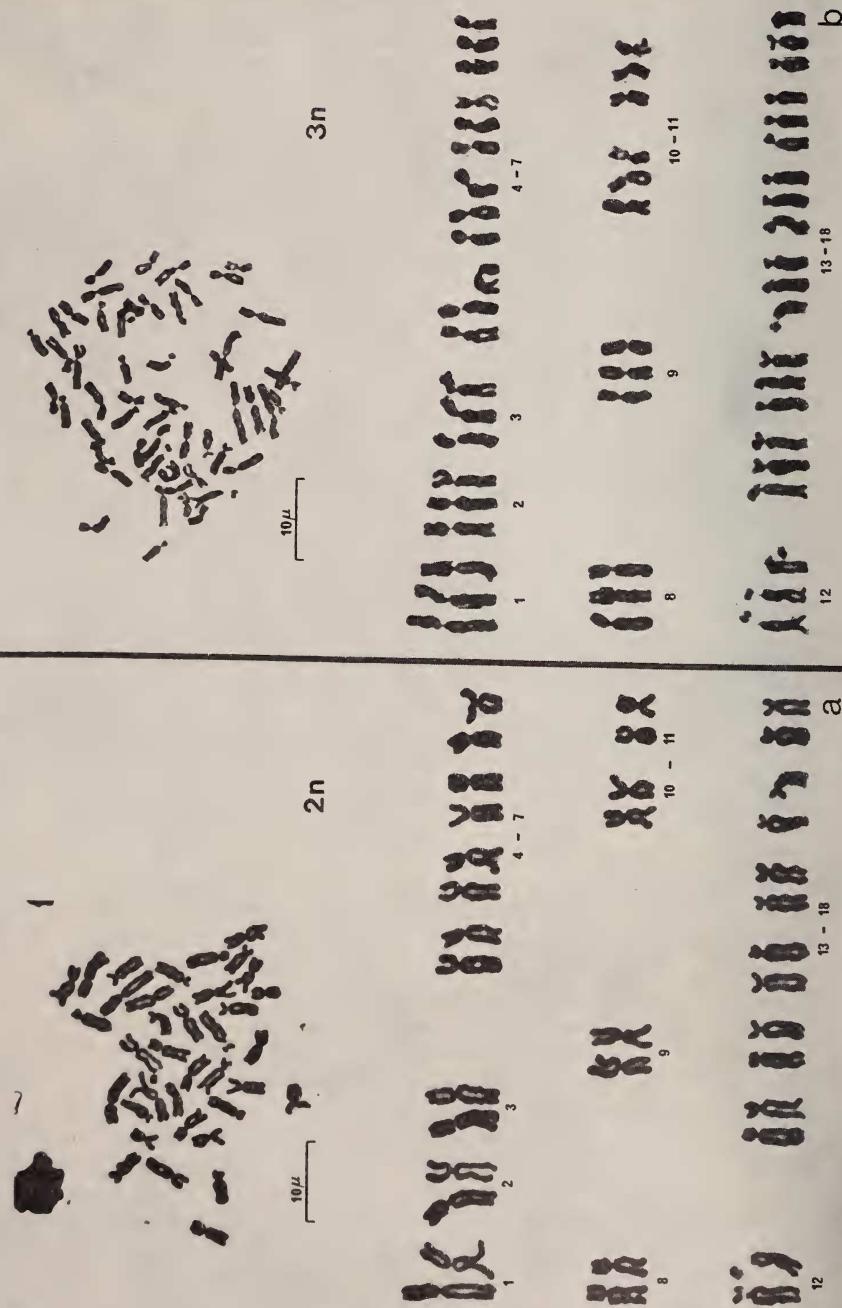


FIG. 3. — Mitoses and karyotypes of *Xenopus laevis*. a) diploid and b) triploid. Acetic orceine  $\times 400$  original.

TABLE I.  
*The effect of pressure treatment*

Pressure	♀	Normals and abnormals										abnormals only						normals only				
		Σ		1 N			2 N		3 N		higher N and mosaics		Σ		3 N		Σ		3 N			
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
290 at	♀1	21	100	—	—	17	81.0	3	14.3	1	4.8	—	—	—	—	21	100	3	14.3	—	—	
	♀2	60	100	—	—	29	48.3	25	41.7	3	5.0	18	100	—	—	42	100	19	45.2	—	—	
	♀3	143	100	5	3.5	49.7	66	46.2	1	0.7	2.3	100	10	43.5	120	100	56	46.7	—	—		
	Σ	224	100	8	3.6	117	52.2	94	42.0	5	2.2	41	100	16	39.0	183	100	78	42.6	—	—	
340 at	♀1	125	100	—	—	16	12.8	94	75.2	15	12.0	20	100	5	25.0	105	100	89	84.8	—	—	
	♀3	189	100	23	12.2	86	45.5	79	41.8	1	0.5	69	100	23	33.3	120	100	56	46.7	—	—	
	Σ	314	100	23	7.3	102	32.5	173	55.1	16	5.1	89	100	28	31.5	225	100	145	64.4	—	—	
	♀1	213	100	2	0.9	1	0.5	196	92.0	14	6.6	29	100	16	55.2	184	100	180	97.8	—	—	
400 at	♀3	184	100	13	7.1	16	8.7	146	79.3	9	4.9	70	100	38	54.3	114	100	108	94.7	—	—	
	Σ	397	100	15	3.8	17	4.3	342	86.1	23	5.8	99	100	54	54.5	298	100	288	96.6	—	—	
	♀3	187	100	1	0.5	—	—	170	90.9	16	8.6	38	100	25	65.8	149	100	145	97.3	—	—	
	Σ	75	100	—	—	75	100	—	—	—	—	4	100	—	—	—	71	100	—	—	—	—
Control	♀1	25	100	—	—	25	100	—	—	—	—	1	100	—	—	—	24	100	—	—	—	—
	♀2	25	100	—	—	25	100	—	—	—	—	3	100	—	—	—	25	100	—	—	—	—
	♀3	25	100	—	—	25	100	—	—	—	—	4	100	—	—	—	22	100	—	—	—	—

as no detailed cytological analysis of the pressure-induced disturbances has so far been made on the treated eggs.

As can be seen from Table 1 the diploid embryos are almost absent at the most effective pressures used (400 and 450 at). Moreover almost all other heteroploid embryos and tadpoles are to be found amongst the abnormals. In order to get a high yield of triploid tadpoles the eggs of *X. laevis* must be exposed to 400 or 450 at pressure 5' after artificial insemination. After elimination of the abnormals one is left with 94.7 to 97.8% of triploids.

We now intend to produce diploid gynogenetic *Xenopus* by pressurizing eggs which have been inseminated with UV-irradiated sperm.

#### ACKNOWLEDGMENTS

Dr. Andrea Brown and Dr. Janina Tymowska provided the triploid and the diploid chromosome preparations respectively. Mr. Alex Portianucha carried out the photography. Mr. Bernard Dumont constructed the pressure chamber and piston.

#### RÉSUMÉ

La triploidie est induite par pression hydrostatique chez *Xenopus laevis*. Cinq minutes après la fécondation artificielle, les œufs sont soumis à la pression pendant 5 minutes. Ce sont des pressions de 400 à 450 at qui donnent les meilleurs résultats. 97% des embryons et des têtards normaux sont triploïdes. Cette méthode est comparée d'une part aux expériences de choc de température sur des œufs de *X. laevis*, d'autre part aux résultats obtenus par pression des œufs chez *Rana pipiens*.

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